Inter-Lab Study Worksheet:

Section I: Provenance & Release

1. Who did the actual work to acquire these measurements?
    - Philipp Popp

2. What other people should be credited for these measurements? (i.e., who would be an author on any resulting publication. For example, your faculty advisor may have helped design the protocols that you ran.)
    - Anna Sommer, Jara Radeck

3. On what dates were the protocols run and the measurements taken? (this will often be a range of dates; make sure you say which data was taken at what times.)
    - Cloning start: 11.09.2014
    - First measurement: 16.09.2014
    - Second measurement: 17.09.2014
    - Third measurement: 18.09.2014

4. Do all persons involved consent to the inclusion of this data in publications derived from the iGEM interlab study?
    - Yes

Section II: Protocol

1. What protocol did you use to prepare samples for measurement?
    - From overnight cultures (14h, 37°C, 200 rpm, 3ml) 50 µl of each construct were mixed with 1 µl FM4-64 then incubated at 37°C for 30 min. From this mixture 1 µl was diluted in 200 µl PBS and then measured by flow cytometry.

2. What sort of instrument did you use to acquire measurements?
    - The flow cytometer is from: BD, model: Accuri c6
    - Containing lasers: blue (488nm) and red (640nm)
    - Emission filters: FL1: 533/30H, FL2: 585/40, FL3: 670LP, FL4: 675/25

3. What protocol did you use to take measurements?
    - Flow rate was set to 10 µl/min; core size: 5 µm; Threshold FSC-A: 11000; time limit: 2 min
    - Samples were gated: (FL3-A > 10^3 FU)

4. What method is used to determine whether to include or exclude each sample from the data set?
    - In case an overnight culture did not grow, it would have been excluded (which was never the case).
    - Data of all three measurements was compared “by eye”.

5. What exactly were the controls that you used?
    - DH5a containing no plasmid with FM4-64
    - PBS without FM4-64
    - PBS with FM4-64

6. What quantities were measured? (e.g., red fluorescence, green fluorescence, optical density)
    - Red fluorescence (FL3-A)
    - Green fluorescence (FL1-A)

7. How much time did it take to acquire each set of measurements?
    - Approximately 45 min

8. How much does it cost to acquire a set of measurements?
    - 2.5 €

9. What are the practical limits on the number or rate of measurements taken with this instrument and protocol?
    - Only one measurement (one sample) at a time, only at specific time points (no continuous measurement)

Section III: Measured Quantities

1. For each type of quantity measured (e.g., fluorescence, optical density), report on the following:
2. Units:
    - Numbers of cells: actual number of FM4-64-dyed cells passing the laser of the flow cytometer in the given measurement time (2 min)
    - FL1-A: Fluorescence units (area of green emission) (correlates with level of GFP expression in a cell measured by excitation with a laser beam)
    - FL3-A: Fluorescence units (area of red emission) (to differentiate between membrane-stained cells and particles)
    - Those fluorescence units could theoretically be described in candela (SI), but we didn't do that and it's not necessary for evaluation

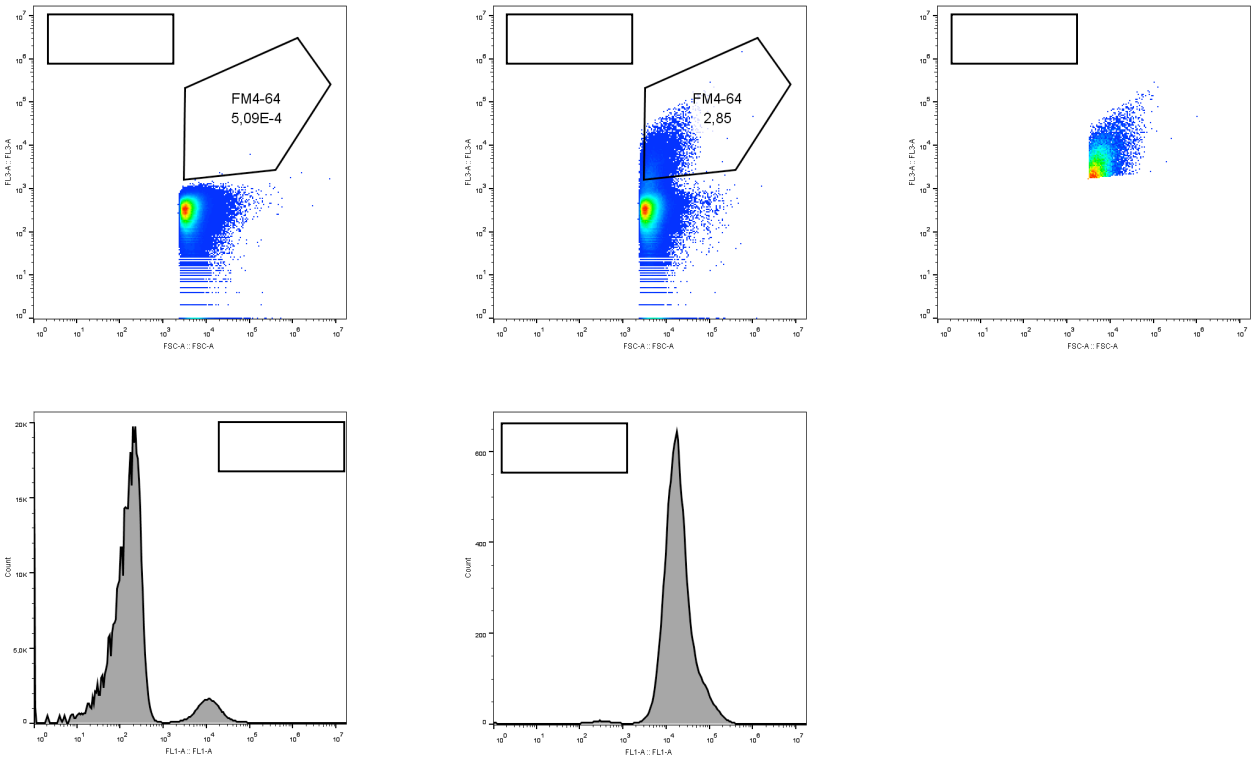
3. Precision:
    - The limit of events per second (=cells per second that pass the laser beam in the flow cytometer) should be less than 5000 to assure that only one cell is passing at a time
    - Fluorescence is measured on a single cell level
    - Range: 0- 16,777,215 Fluorescence units (FU) for each channel, significance: 1
    - Presumably the precision is the same across the entire range
    - Data sheet from the manufacturer

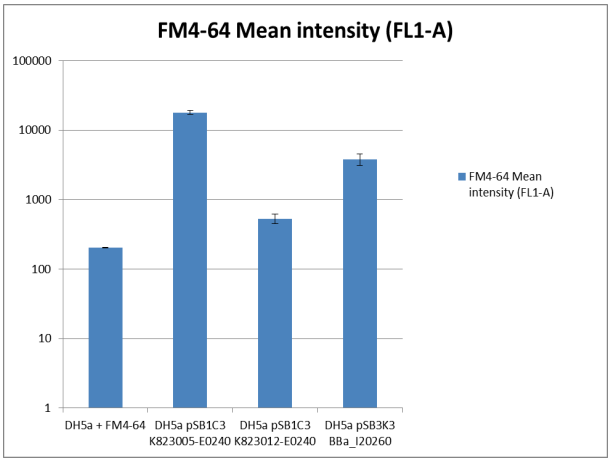
4. Accuracy:
    - Calibration was performed in June 2014 with (6peak/8peak beads) and confirmed by the manufacturer
    - To provide the possibility to compare our data with data from other flow cytometers, we included those beads on two day of the measurements (settings: "slow" flow rate, 50,000 events)

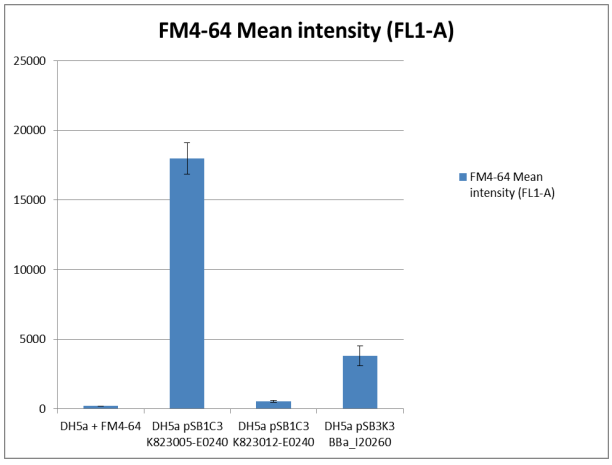
Figure 3

Section IV: Measurements

- Figure 1: Setting gate for red labeled cells (PBS with FM4-64)
- Figure 2: Cells dyed with FM4-64 (calls are in the gate, dust is outside)
- Figure 3: Cells for evaluation (only gated events (=cells) are shown)
- Figure 4: Histogram showing GFP-fluorescence units (FU) before gating
- Figure 5: Histogram showing GFP-fluorescence units (FU) after gating
- Figures 6+7: Geometric mean of GFP-fluorescence for each strain (log & linear scale); error bars indicate standard deviation of the geometric mean (3 measurements)
- Table 1: measurements
- Table 2: summary of measurements







ıble 2

Measurement	Date	Sample	Total event number	FM4-64 Gate event number	FM4-64 Mean intensity (FL1-A)	FM4-64 CV intensity (FL1-A) = coefficient of variance
1	16.09.2014	PBS	589161	3	6861	0
1		DH5a pSB1C3 K823005-E0240	539022	15369	17040	78,1
1		DH5a pSB1C3 K823012-E0240	53213	8672	571	54,4
1		DH5a pSB3K3 BBa_I20260	74891	18946	4799	68,7
2	17.09.2014	PBS	30835	1	48787	NA
2		PBS + FM4-64	10837	50	286	237
2		DH5a + FM4-64	68783	30979	207	68,4
2		DH5a pSB1C3 K823005-E0240	202794	103877	19563	59,5
2		DH5a pSB1C3 K823012-E0240	114885	36408	607	50,5
2		DH5a pSB3K3 BBa_I20260	152288	39865	3525	60,3
3	18.09.2014	PBS	75720	1	466	NA
3		PBS + FM4-64	81110	116	160	112
3		DH5a + FM4-64	292767	96751	200	75,6
3		DH5a pSB1C3 K823005-E0240	303137	95623	17390	66,3
3		DH5a pSB1C3 K823012-E0240	275651	83511	416	55,2
3		DH5a pSB3K3 BBa_I20260	291692	85840	3146	52,7

Sample	FM4-64 Mean intensity (FL1-A)	SD of the mean
PBS	18704,7	
PBS + FM4-64	223,0	63
DH5a + FM4-64	203,5	3,5
DH5a pSB1C3 K823005-E0240	17997,7	1116,0
DH5a pSB1C3 K823012-E0240	531,3	82,9
DH5a pSB3K3 BBa_I20260	3823,3	707,0

We can provide the original FCS-files or csv-files, if requested.